Effect of Some Heterocyclic Compounds on Human Serum AChE

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Abstract

This study is designed to show the effect of fifteen heterocyclic compounds on the activity of AChE in sera, all compounds showed strong inhibition ranging between (80.3 %-93.7%) at concentration up to 1x 10^{-2} M.it was found that the percentage of inhibition is increased with the increase of concentration of inhibitors and 3-phenyl-6-[5'nito-2'-furyl)ethyl]-1,2,4-triazole[3,4,6], [1,3,4] thiodiazole (9) was the most effective inhibitors. Kinetic properties of these inhibitors are studied and Lineweaver-Burk plots were nused to determine the type of inhibition, all studied compounds behaved as non-competitive inhibitors. The mechanism of action of heterocyclic compounds as inhibitor to serum AChE activity was suggested.

الخلاصة

تم في هذا البحث دراسة خمسة عشر من المركبات الحلقية غير المتجانسة على فعالية انزيم الاسيتايل كولين استريز في مصل دم الانسان حيث اظهرت المركبات المدروسة تاثير تثبيطي قوي على فعالية الانزيم و ان نسبة التثبيط تزداد مع زيادة تركيز المثبطـات حيـث تـرواحـت النسـبة المؤية للتثبيط بين 80.3 % - 93.7 % بأستخدام التركيز 1*10^{-2} مولاري.

و قد لوحظ ان للمركب ترايازول [6,4,3] , [4,3,1] ثايادايازول (9) اعلى قابلية لتثبيط الانـزيم من بقية المركبات, كذلك تمت دراسة الخواص الحركية لهذه المركبات حيث اظهـرت المركبـات المدروسة انها تسلك سلوك مثبطات غير تنافسية. لقد تم في هذه الدراسة ايضا اقتراح الية تثبـيط لعمل هذه المركبات على انزيم الاسيتايل كولين استريز.

Introduction

Heterocyclic compounds are widely distributed in anature and are essential to life, they play a vital role in the metabolism of all living cell as carbohydrate, proteins and enzyme [1]. There are a vast number of pharmacological active heterocyclic compounds, many of which are in regular clinical use. Many heterocyclic compounds are insecticides, because of there activity as nerve poisons inhibiting cholinesterase enzyme [2].

A number of heterocyclic are endowed with a large number of biological and pharmacological activities, such as antimicrobia, antifungal [1], insecticidal [2], virucidal [3], acaricidal [4] anti-inflammatory and central nervous system [5,6]

Furthermore, some thiadiazole compounds have been found to exhibit antibacterial, antiviral, and anticancer activities [7], and some compounds have shown to possess analgesic properties and exhibit a marked activity in the control of bacterial growth [8]. Some biological properties of thiadiazoles are worthy of consideration because of their activities as fungicides, parasiticides and amebicides [9].

Moreover, some thiadiazol compounds are reputed for exhibiting moderate tumor inhibitory properties as well as their uses as anti tubercular [1], anti carcinogenic [20], and muscle relaxant agents [11].

Investigators have demonstrated that some triazole compounds exhibit fungicidal properties [12,13], and some are useful as anti inflammatory, cardiotonic and herbicidal agent [14].

Some triazole compounds have been found to exhibit high activity against ten selected HIV mutants [15], and other triazole compounds have been also employed as antibacterial and antifungal agents[16,17] Number of triazole compounds are endowed with large number of medical and biological activities such as tuberculostatic[18] and antimicrobial[19,20].

Experimental

Different heterocyclic compounds were examined, regarding their effects on the activity of AChE. The solubility of these compounds in dimethyl sulfoxide (DMSO) solvent, and the stock solution of each compound (0.5M) were prepared. The effect of the solvent DMSO on the activity of AChE was tested and did not show any inhibitory effect [14]. The percentage of inhibition was calculated at different concentrations ($1X10^{-2} – 1x10^{-8}$) M for each compound. The different concentrations of the compounds were prepared by dilution with DMSO using the stock solution.

Procedure:

AChE activity was measured in human senim using the modified Ellman method [15] as follows:

(50 µl) of DTNB solution (0.001 M) was added to 2.25 ml of phosphate buffer solution (l ml of inhibitor mixed with 1.25 ml of buffer (pH=7.3, 0.2 M), then (10µl) of serum was added, mixed well and (2 ml) of the mixture was transferred to a measuring cell (3 mm), then (34 µl) of ASChI (0.06M) was added, the change in absorbency was measured before and after'adding the substrate at (430 nm) for (3 min).

The inhibition percentage was calculated by comparing the activity with and without the inhibitor and

under the same conditions, according the equation:

$$\% \text{ Inhibition} = 100 - \left(\frac{\text{The activity in the presence of inhibitor}}{\text{The activitv in the absence of inhibitor}}\right) * 100$$

Study of Inhibition Type:

A constant concentration of inhibitor was used with different substrate concentrations ranging from (0.02 - 0.09 M). These different cone, were prepared from the stock solution of (0.l M) ASChl. Two concentrations ($1x10^{-4}$, $1x10^{-6}$) M of compounds (2, 3, 4, 8, 10, 11, 12, 13) and ($1x10^{-5}$, $1x10^{-6}$) *M* of compounds (1, 5, 6, 7, 9, 14, 15) were used.

The enzyme activity was determined with and without the inhibitor. Using the line weaver- Burk method by plotting 1/v vs. 1/[S]V the following values were calculated:

1) Ki value.

2) Vmapp.

3) Type of inhibition

Results and Discussion

The present work is designed to investigate the biological activity and effects of different series of heterocylic compounds (table 1) on human serum AChE activity in vitro.

According to the results obtained the compounds could be Classified into four series as shown below:-

(i) Series (I) included 1, 3, 4- Thiadiazole derivatives which are compounds (1, 2, 12, 13) as shown in table (1).

(ii) Series (II) included 1, 2, 4- Traizole derivatives, which are compounds (3, 4, 5, 6, 7, 8, 10, 11) in table (1).

(iii) Series (III) included the substituted benzoic acid hydrazone containing 5- nitro furan moiety, this series is included compounds (14,15) in table (1).

(iv) Series (IV) include compound (9) in table (1) and compound 3- phenyl- 6- (5- nitro- 2- furyl) ethenyl]- 1, 2, 4- triazole [3, 4, 6] [1, 3, 4] thiodiazole.

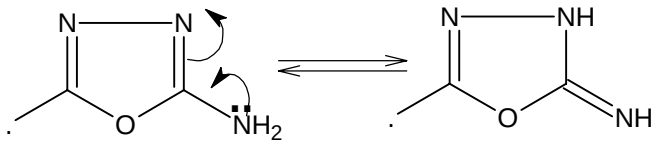
All compounds in these series (I- IV) showed inhibitory effect on the enzyme activity and the relationship between inhibitor concentration and percentage of inhibition are shown in figures (1-4).

The enzyme activity decreased with increased inhibitor concentration until reaches the minimal value. The highest inhibition of each compound was observed at concentration (1 * 10"^{2}M) as shown in fig. (5).

In order to understand the mechanism of inhibition of these compounds, the structural properties of each compound within series were compared according to the results in figs. (1-5)and the following findings were observed. (1) Series (I) compounds (1) and (2) exhibited higher percentage of inhibition (89 and 87.5%) respectively than the other two compounds (12 and 13) which exhibited lower percentage of inhibition (84.1% and 80.3%) respectively. This could be attributed to the presence of 5- nitrofuryl moiety in compounds (1) and (2), in addition, of substituted phenyl group in 1, 3, 4- thiodiazole ring.

These groups enhanced the nucleopholcity of $\diagdown C = N$ attack the binding site of inhibitor subsequently, decreased the activity while in compounds (12) and (13), 5- nitrofuryl moiety were absent, so the NH_2 group may attack the active site of enzyme but not as in compounds (1) and (2).

This may be caused the delecolization of the pair of electrons in NH_2 via thiadiazol ring.



(2) Series (II) compounds inhibition can be divided into two groups according to their structures:-

Group which have no 5- nitrofuryl moiety in its structure and includes compounds (10) 4- amino- 1, 2, 4-triazole and compound (11) 3,5- diphenyl- 4- amino 1, 2, 4-traizol. 3, 5- diphenyl- 4- amino 1, 2, 4- triazol shows higher percentage of inhibition (93.5%) than 4- amino-1, 2, 4- traizole which could be attributed to the presence of two phenyl groups at position 3 and 5, this conjugation of phenyl group wmv $\diagdown C = N$ made its availability as electron rich weaker than free NH_2 group at position 4 to attack the active site of enzyme.

Group £b) which have 5- nitrofuryl moiety in its structure and includes compounds:-

(3) 5- Amino 2- mercapto- 4- [5'- nitro- ***2 '*** - furyl] methylene amino, 4H- 1, 2, 4- triazole.

(4) 3- Mercapto- 5- (p- $N0_2$ phenyl)- 4H- 1, 2, 4, traizole.

(5) 5- Amino- 3- mercapto- 4 [3'-(- 5'- nitro- 2- furyl) prop- 2 - enylidene amino]- 4H 1, 2, 4- traizole.

(6) 5- Amino- 3- hydroxy- 4 [(3'-[- 5'- nitro- 2- furyl] prop- 2 enylidene) amino]- 4H 1 , 2 , 4- traizole.

(7) 3- mercapto- 4- phenyl- 5- [(3'- [5'- nitro- *T* - furyl] prop-2 enyliden) amino] 4H 1 , 2 , 4- traizole.

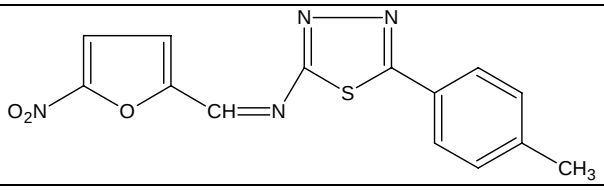
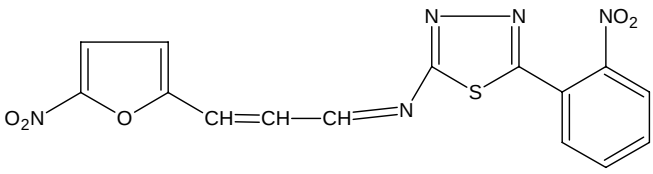
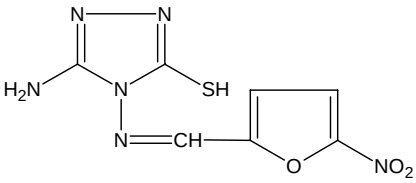
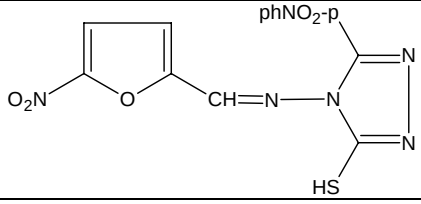
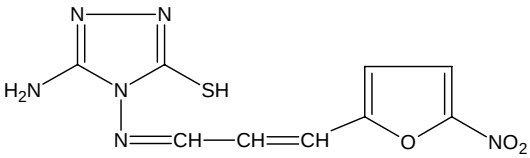
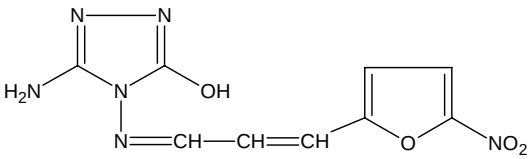
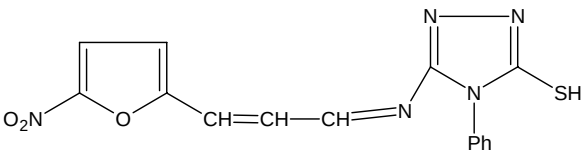
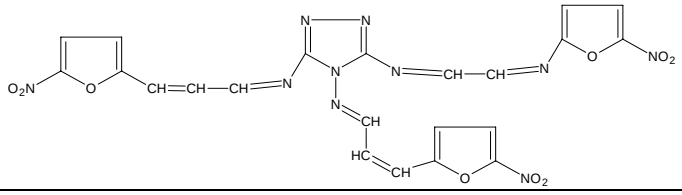
(8) Tri [3-(5'- nitro- *T* - furyl) prop 2 enylidene) amino]-4H-1, 2, 4- triazole.

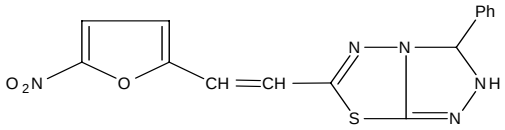
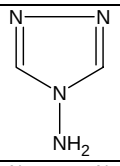
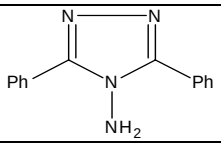
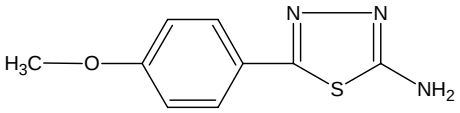
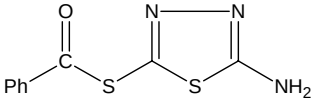
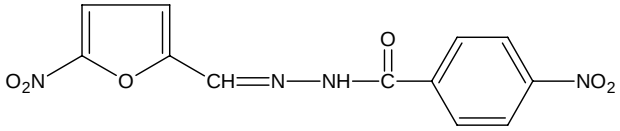
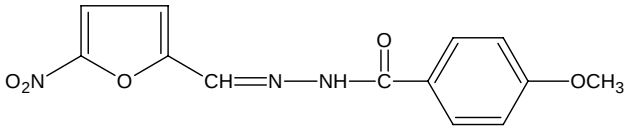
Generally, The compounds of this group show higher percentage of inhibition ranged between (81.8-92.3%).

Compound (7) shows relatively higher percentage of inhibition than compounds (8) and (4), which may be attributed to the groups that substituted at positions 4 and 5. This make the electron rich side in this compound (SH or $\diagdown C = N$) easier to approach the binding site of inhibitor. Furthermore, the binding site of inhibitor is relatively less steric than others.

Compound (3) is more potent inhibitor than compound (5) in spite of the similarity of the two compounds in structure, because compound (5) has long chain (conjugation) at position 4 than compound (3) which causes decrease in the ability to attack the binding site of inhibitor, while the inhibitory effect of compound (6) is also relatively higher than compound (6) which may be attributed to the high nucleophilicity of OH group at position 3 in compound ***(6),*** though, the presence of other nucleophilic side (e. g. NH_2) in both of them.

Table 1-: Heterocyclic Compounds used for interaction with AChE

Comp. No.	Structure	Name	Mwt.
1		5-(p-methyl phenyl)-3-[5'-nitro-2'-furyl] methylamino]-l,3,4-thiodiozole	314
2		5-(o-nitro phenyl)-3-[3(5'-nilro-2'-furyl)Prop-2-enylidene amino)]-1 ,2J,4-thiodizole	326
3		5-Amino-2-mcrcapto -4-[5'-nilro-2'-furyl)methylcne amino)]-4H-1,2,4-triazole	254
4		3-Mercapto -5-(p-N0₂ phenyl)-4H-1,2,4-triazole	238
5		**5-Amino-3-mercapto -4- [3-(5'-nitro-2-furyl)Prop-2-methylene amino)]-4H-l,2,4-triazole**	219
6		**5-Amino-3-hydroxy -4-[3'-(5'-nitro-2-furyl)Prop-2-enylidene amino)]-4H-1,2,4-triazole**	264
7		**3-Mercapto-4-phenyl -5-[3-(5-nitro-2'-furyl)Prop-2-enylidene) amino]-4H-l,2,4-triazole**	341
8		**Tri[3(5-nitro-2'-furyl) Prop-2-enylidene) amino]-4H-l,2,4-triazole**	574

9		**3-phenyl -6-[5'-nitro-2'-furyl) ethyl]-l,2,4-triazolc[3,4,6],[1,3,4] thiodiazole**	339
10		4-Amino-**1**,2,4-Triazole	84
11		3,5-diphenyl-4-amino-l,2,4-triazolc	236
12		2-(amino-5-(p-mcthoxy phenyl)1,3,4-thiadiazole	206
13		2-(amino-5-thio benzoyloxy-1,3,4-thiadiazole	237
14		5-Nitrofurylidene -N-p-N0₂-benzoic acid hydrizde	304
15		5-Nilrofurylidcne-N-p-methoxy-benzoic acid hydrizde	289

Series (III), compounds (14) and (15) are very similar in their structures with the exception of the difference in substituent group at position 4 of phenyl ring, $N0_2$ and OCH_3 respectively. However, $N0_2$ group withdraws electrons from amino group and causes increase of the electrophilic characters of the $\rangle C = N$ toward the binding site of inhibitor.

Series (IV) compound (9) have various $\rangle C = N$ group in different position, therefore its ability to attack the active site of enzyme is high.

Type of Inhibition

Type of inhibition, K_{mapp}, V_{mapp} and Kj were estimated by measuring the enzyme activity in the absence and presence of inhibitor at different concentrations of substrate under the same conditions using Lineweaver- Burk equation and plot, as shown in table (2) and fig (6-20).

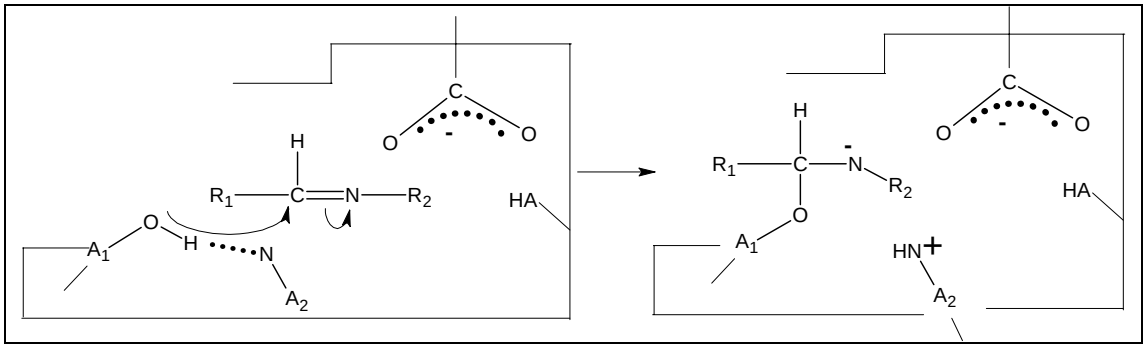
The results suggested that compounds 1-15 are non-competitive inhibitors.

In non competitive type of inhibition which does not effect the combination of substrate with the enzyme, Km remained constant while V_{max} change dramatically.

Mechanism of Inhibition

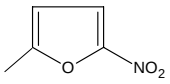
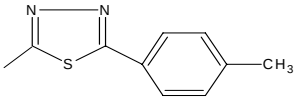
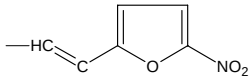
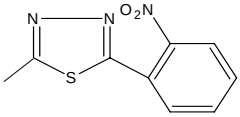
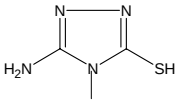
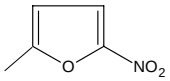
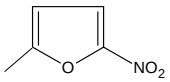
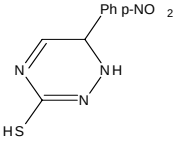
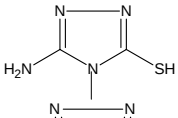
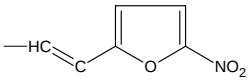
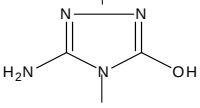
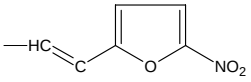
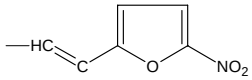
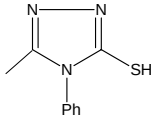
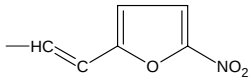
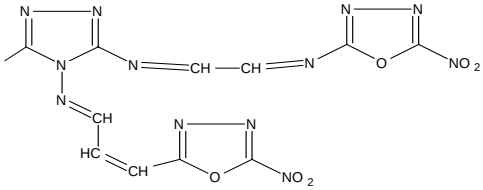
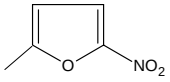
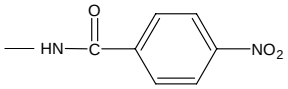
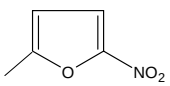
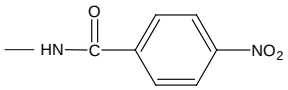
In the mechanism of hydrolysis ACh by AChE, the hydroxyl group of the amino acid serine is connected with the imidazole of the histidene residue via hydrogen bonding which causes increase of the nucleophilic characters of hydroxyl group to form a covalent bond with the carbonyl carbon of acetylcholine as in scheme (1) to form enzyme-substrate complex, after that the choline molecule is released and latter the hydrolysis takes place to produce acetic acid and regenerate free enzyme [24,24,26].

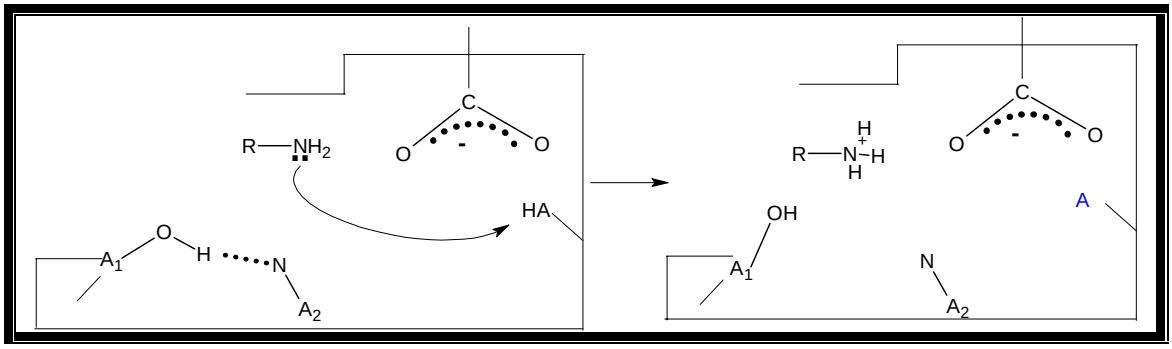
The proposed mechanism of action of heterocyclic compounds as AChE inhibitors studied as indicated are shown in schemes (2-5) with the consideration that the inhibitor binding site is in close resemblance with the active site functional groups.



Scheme(2) Suggested mechanism for inhibition AChE by compounds (1,2,3,4,5,6,7,8,14, and 15)

A_1= Ser, Glu, Asp, and Tyr **A_2= His, Lys, Arg, Gln, and Asn**

	R_1	R_2
1		
2		
3		
4		
5		
6		
7		
8		
14		
15		

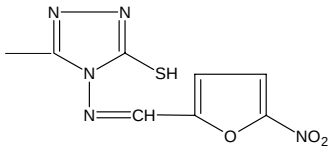


Scheme(3) Suggested mechanism for inhibition AChE by compounds (3,5,6,10,11,12, and 13)

A_1= Ser, Glu, Asp, and Tyr **A_2= His, Lys, Arg, Gln, and Asn**

R

3



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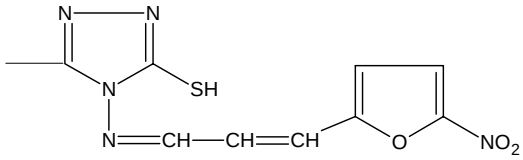

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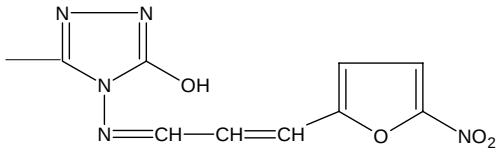

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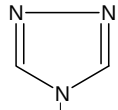

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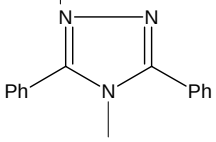

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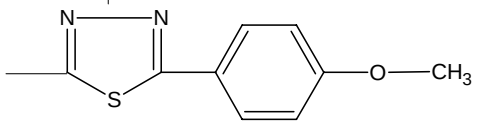

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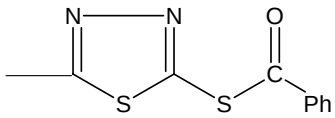
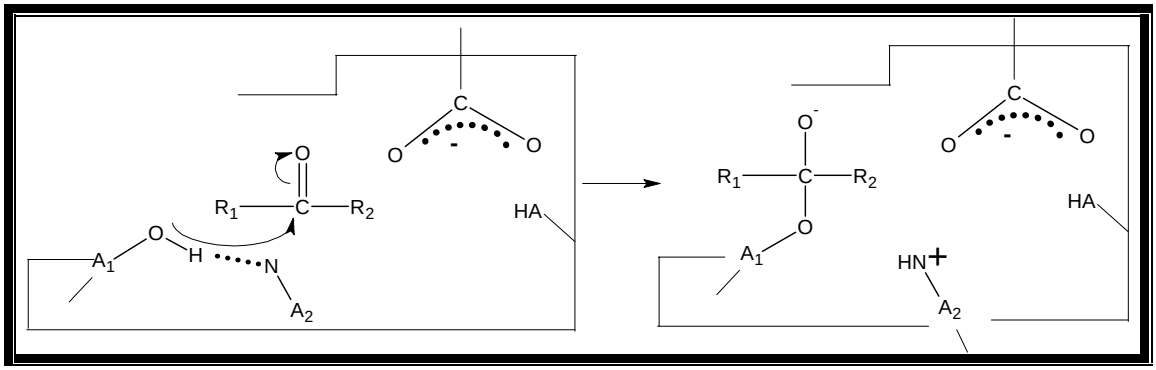
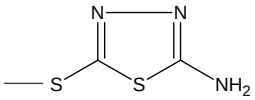
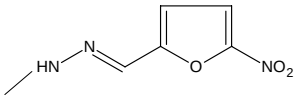
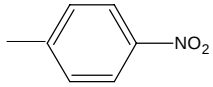
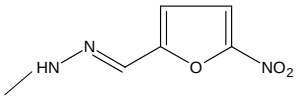
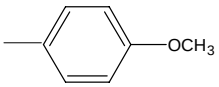
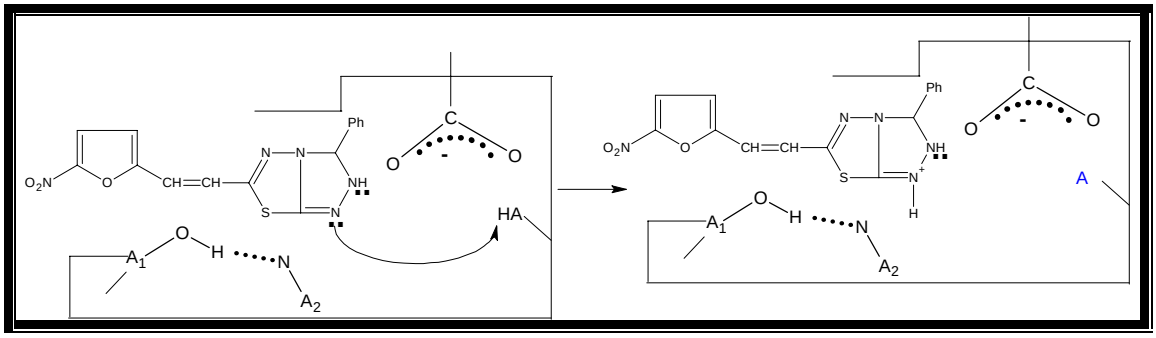




Scheme(4) Suggested mechanism for inhibition AChE by compounds (13,14, and 15)

A_1= Ser, Glu, Asp, and Tyr **A_2= His, Lys, Arg, Gln, and Asn**

	R_1	R_2
13	Ph	
14		
15		



Scheme(5) Suggested mechanism for inhibition AChE by compound (9)

A_1= Ser, Glu, Asp, and Tyr **A_2= His, Lys, Arg, Gln, and Asn**

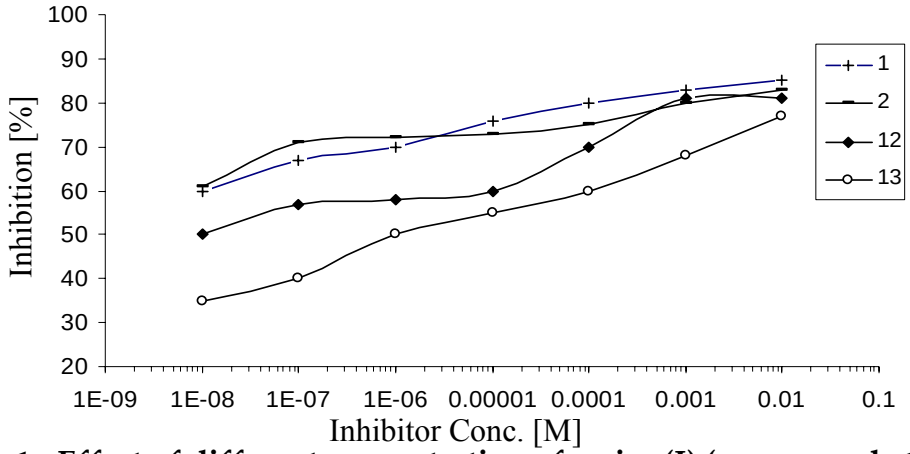


Fig. 1-: Effect of different concentration of series (I) (compounds 1, 2, 12, and 13) on human serum AChE activity

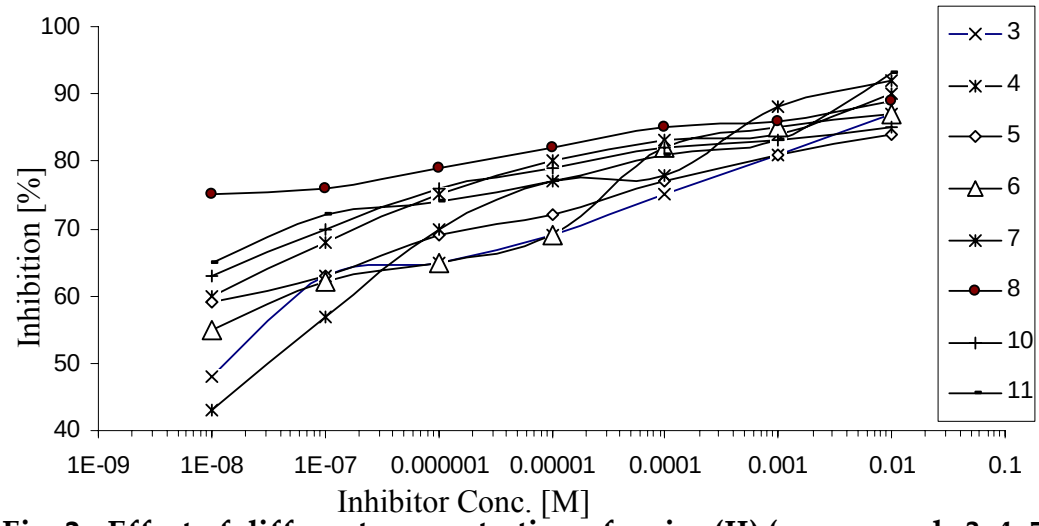


Fig. 2-: Effect of different concentration of series (II) (compounds 3, 4, 5, 6, 7, 8, 10, and 11) on human serum AChE activity

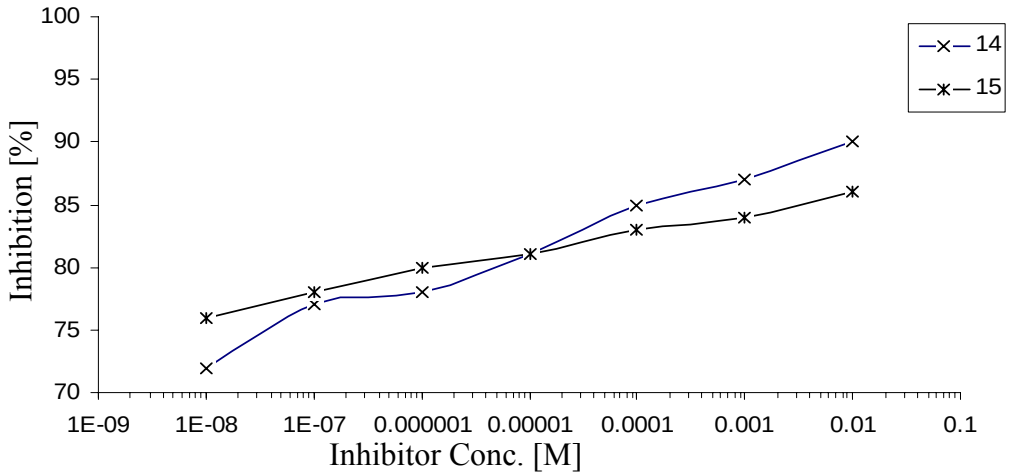


Fig. 3-: Effect of different concentration of series (III) (compounds 14 and 15) on human serum AChE activity

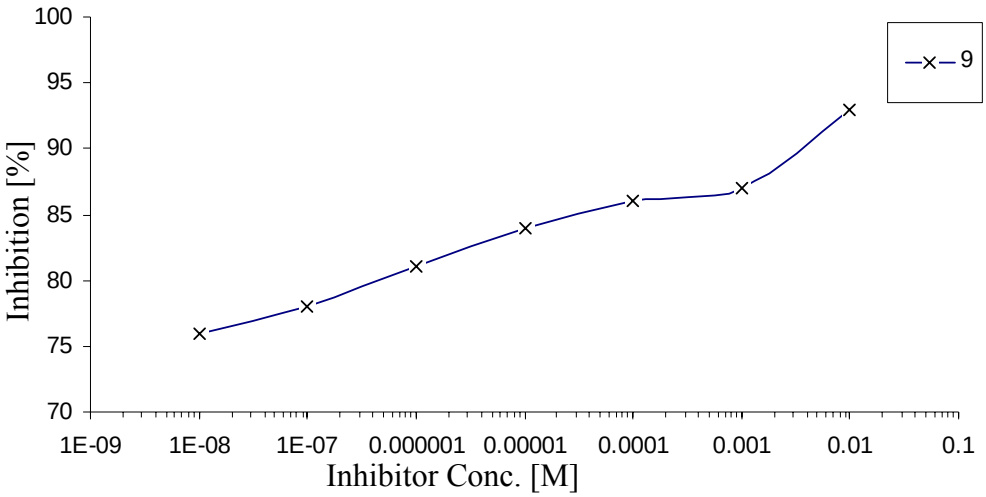


Fig. 4-: Effect of different concentration of series (IV) (compound 9 on human serum AChE activity

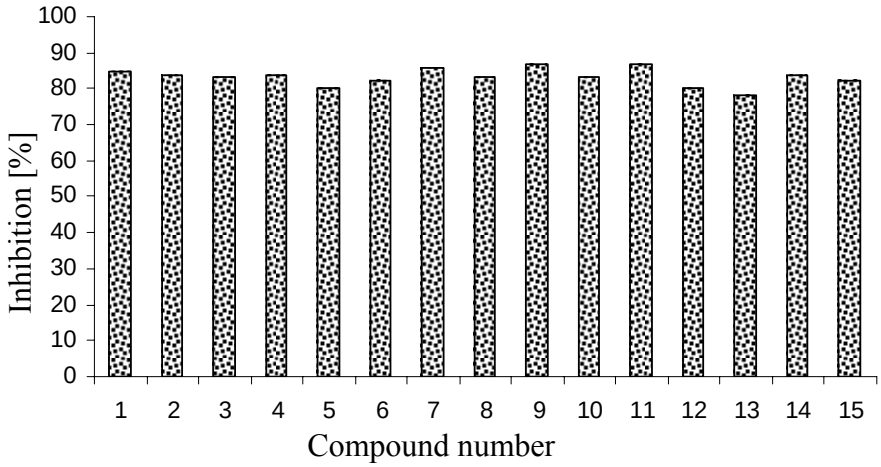


Fig. 5-: Diagram shows the effect of 10^{-2} M of compound (1-15) on human serum AChE activity.

Table (2)Kinetic Parameter of AChE with different heterocyclic compounds

Comp. No $1x10^{-4}$M	**Vmapp** (jimoie/ **ml/ min)**	Ki(M)
1	2.169	2.29×10^{-5}
2	2.33	2.69×10^{-5}
3	2.345	2.36×10^{-5}
4	2.55	2.69×10^{-5}
5	2.62	2.43×10^{-5}
6	3.02	2.17×10^{-5}
7	2.234	2.97×10^{-5}
8	2.63	2.46×10^{-5}
9	3.16	2.78×10^{-5}
10	2.48	2.85×10^{-5}
11	2.817	2.96×10^{-5}
12	2.38	1.88×10^{-5}
13	2.91	2.46×10^{-5}
14	2.75	2.57×10^{-5}
15	3.209	2.22×10^{-5}

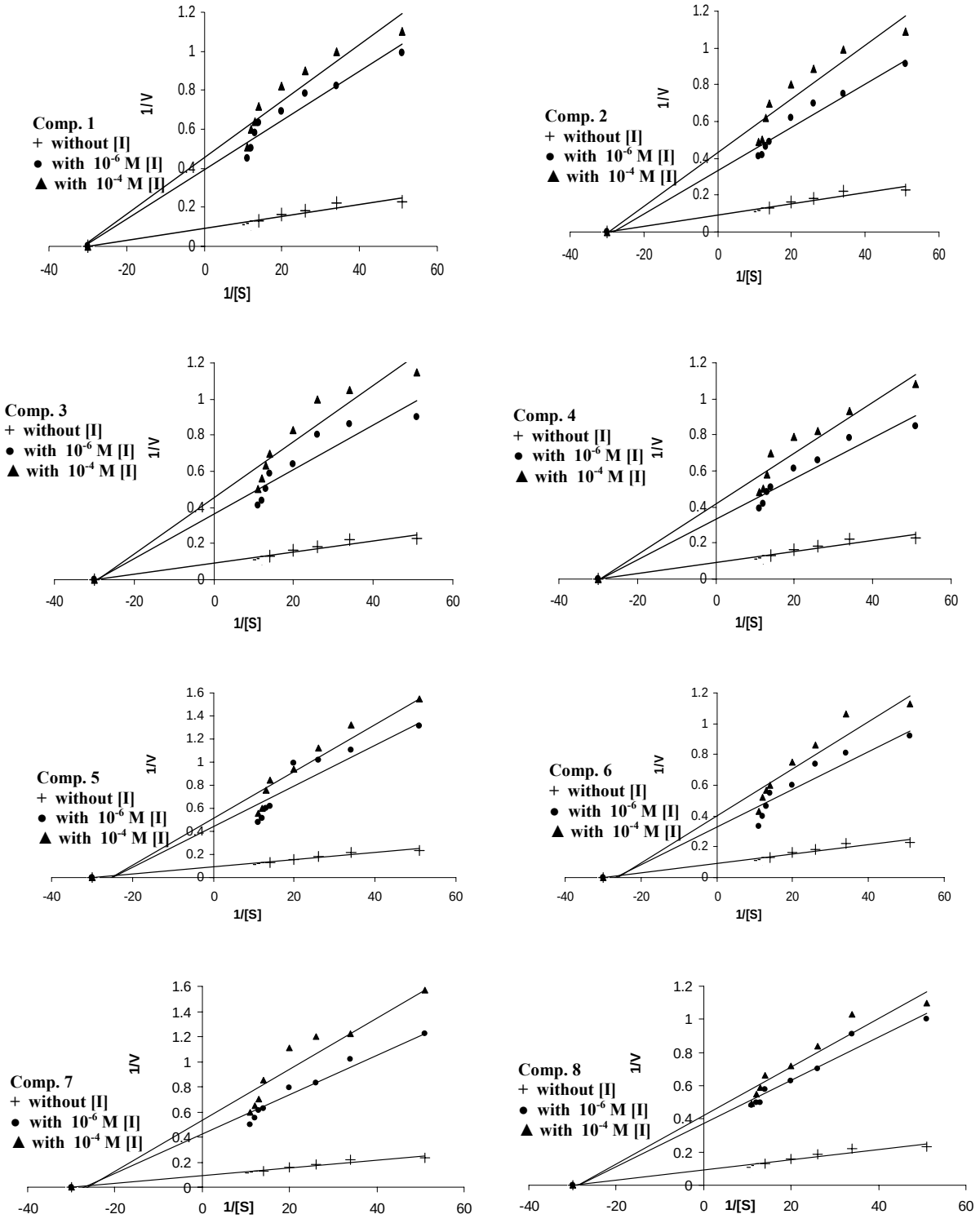


Fig. -6: Line Weaver-Burk plot of AChE in the presence and absence of compounds 1-8

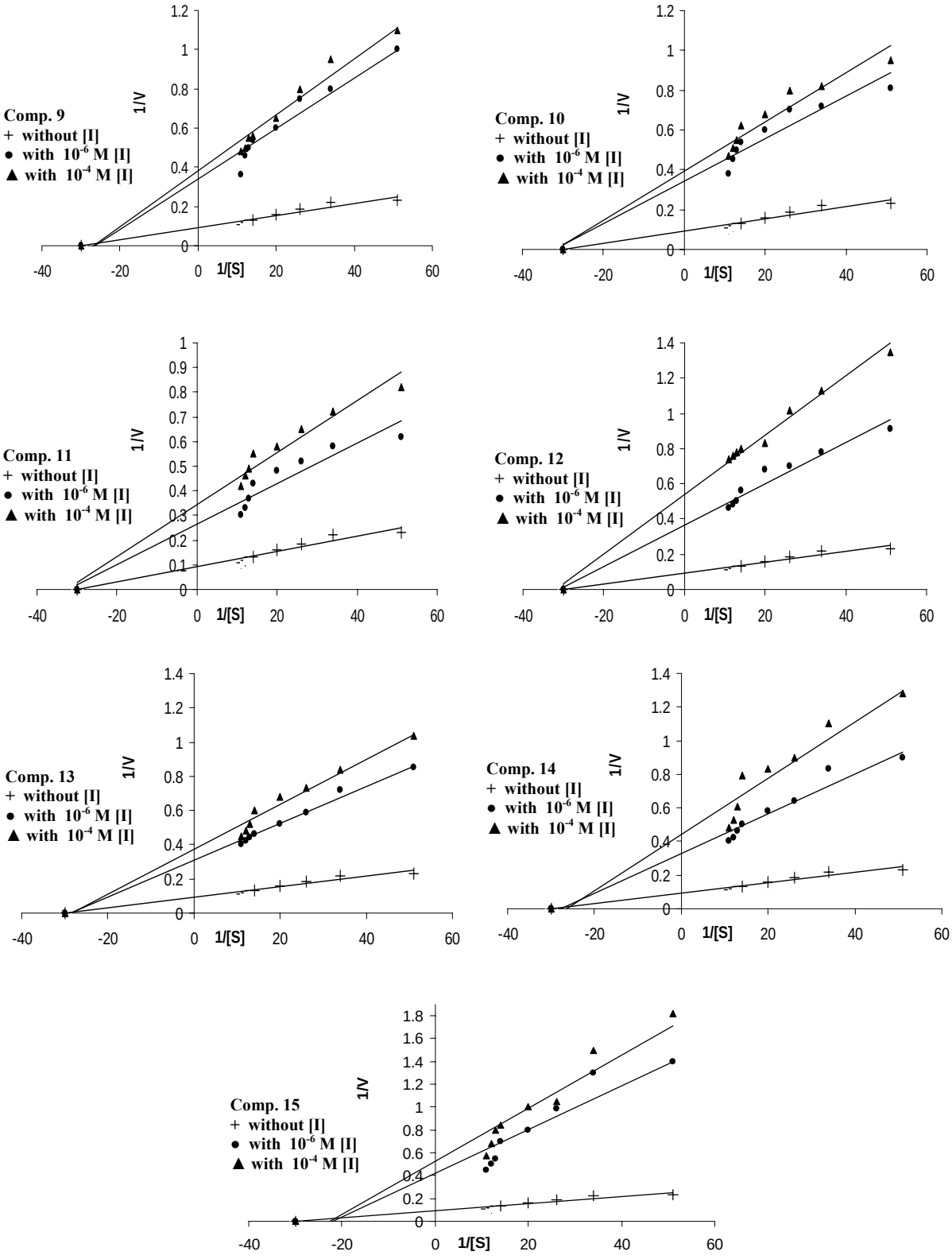


Fig.-7:Line Weaver-Burk plot of AChE in the presence and absence of compounds 9-15

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